

# Genome Sequence of *Bradyrhizobium japonicum* E109, One of the Most Agronomically Used Nitrogen-Fixing Rhizobacteria in Argentina

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**We present here the complete genome sequence of *Bradyrhizobium japonicum* strain E109, one of the most used rhizobacteria for soybean inoculation in Argentina since the 1970s. The genome consists of a 9.22-Mbp single chromosome and contains several genes related to nitrogen fixation, phytohormone biosynthesis, and a rhizospheric lifestyle.**

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The soybean-*Bradyrhizobium* symbiosis is considered one of the most efficient in fixing N<sub>2</sub> and probably the greatest in economic importance around the world (1). The agronomic cultivation of soybeans (*Glycine max* L.) affects >20 million ha in Argentina, where at least 85%, on average, are biologically treated with bradyrhizobia (2). In the 1970s, several strains belonging to the genus *Bradyrhizobium* were received in Argentina from different collections around the world to be evaluated under agronomical conditions, including strain 2860 (previously named USDA138 and corresponding to the *Bradyrhizobium japonicum* USDA6 serogroup), sent by C. N. Hale from the Department of Scientific and Industrial Research (DSIR) in New Zealand. After evaluation, selection, and reisolation from soybean nodules, the strain was renamed E109. To date, *B. japonicum* E109 has been the only strain recommended by the Instituto Nacional de Tecnología Agropecuaria (INTA) for soybean inoculation due to its capacity to effectively colonize the plant and fix nitrogen, increasing crop productivity (3). Together with this ability, alternative mechanisms have been proposed to explain the growth promotion, especially in nonlegumes, such as phosphate solubilization (4), siderophore production (5), systemic resistance induction (6), and phytohormone biosynthesis (7–9).

We announce here the complete annotated genome sequence of *B. japonicum* E109. The sequence was obtained using a combined whole-genome shotgun and 8-kb paired-end strategy with a 454 GS FLX Titanium pyrosequencer at the Instituto de Agrobiotecnología Rosario (INDEAR) (Argentina), resulting in a 24-fold genome coverage. The sequencing reads were *de novo* assembled (Newbler version 2.9), resulting in 142 contigs ordered in 4 scaffolds (>737 kbp each; N<sub>50</sub>, 4,081,299 bp). Intra- and interscaffold gap closures were achieved by a detailed observation of the relevant sequencing reads using the Geneious R7 software platform (10). The absence of plasmids is a common feature of *Bradyrhizobium* genomes (11–13), and in agreement with the bioinformatics

data, pulsed-field gel electrophoresis (PFGE) analysis of total DNA revealed the presence of a unique chromosome. The genome size is 9,224,208 bp, and the G+C content, in agreement with this bacterial species, is 63.6%.

Genome annotation was done using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) (14). The complete genome consists of 8,233 protein-coding sequences. Similarly to other species of the *Bradyrhizobium* genus, *B. japonicum* E109 contains two identical and complete ribosomal operons. A total of 54 tRNA genes representing 45 tRNA species were identified. In agreement with the genome sequence of USDA6, the presence of a 645-kb DNA region with low G+C content (59.0%) was revealed to be reminiscent of a symbiotic island, which includes most of the *nod*, *nif*, and *fixes* genes. The putative genes involved in other plant growth-promoting mechanisms, such as phytohormone production, were determined using the RAST annotation server (15) and KAAS (16).

The *B. japonicum* E109 genome contains genes related to type II and VI secretion systems, nitrogen fixation, phytohormone biosynthesis, and a rhizospheric lifestyle. The genome sequence of E109 provides a genomic basis for in-depth comparative genome analyses to elucidate the specific mechanisms of *Bradyrhizobium*-plant interactions.

**Nucleotide sequence accession number.** The complete genome sequence of *B. japonicum* E109 is available at NCBI GenBank under the accession no. [CP010313](https://www.ncbi.nlm.nih.gov/nuclot/CP010313).

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## REFERENCES

- Hungria M, Mendes IC. 2014. Nitrogen fixation with soybean: the perfect symbiosis? In: de Bruijn F (ed), Biological nitrogen fixation. Wiley-Blackwell, Hoboken, NJ.
- Piccinetti C, Arias N, Ventimiglia L, Díaz Zorita M, Murua L, Sánchez H, Ferraris G, Mousegne F, Fontanetto H, Sá Pereira E, Capurro J, Enrico JM, López C, Carrizo AS, Salvaggiotti F, Collino D, Perticari A. 2013. Positive effects of inoculation of soybean on nodulation BNF and the parameters of crop production, p 283–297. In: Albanesi AS (ed), Microbiología agrícola: un aporte de la investigación en Argentina, 2nd ed. Magna Publicaciones, Tucumán, Argentina.
- Perticari A, Parra R, Balatti P, Fiqueni M, Rodríguez Cáceres E. 1996. Selección de cepas de *Bradyrhizobium japonicum*, *B. elkanii* y *Sinorhizobium fredii* para la inoculación de soja, p 103–104. Memorias de la XVIII Reunión Latinoamericana de Rizobiología, 23 al 27 de septiembre 1996, Santa Cruz de La Sierra, Bolivia.
- Halder AK, Mishra AK, Chakrabarty PK. 1991. Solubilization of inorganic phosphates by *Bradyrhizobium*. Indian J Exp Biol 29:28–31.
- Chabot R, Antoun H, Cescas MP. 1996. Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. Plant Soil 184:311–321. <http://dx.doi.org/10.1007/BF00010460>.
- Cartieaux F, Contesto C, Gallou A, Desbrosses G, Kopka J, Taconnat L, Renou JP, Touraine B. 2008. Simultaneous interaction of *Arabidopsis thaliana* with *Bradyrhizobium* sp. strain ors278 and *Pseudomonas syringae* pv. tomato DC3000 leads to complex transcriptome changes. Mol Plant Microbe Interact 21:244–259. <http://dx.doi.org/10.1094/MPMI-21-2-0244>.
- Boiero L, Perrig D, Masciarelli O, Penna C, Cassán F, Luna V. 2007. Phytohormone production by three strains of *Bradyrhizobium japonicum*, and possible physiological and technological implications. Appl Microbiol Biotechnol 74:874–880. <http://dx.doi.org/10.1007/s00253-006-0731-9>.
- Cassán F, Perrig D, Sgroi V, Masciarelli O, Penna C, Luna V. 2009. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109 promote seed germination and early seedling growth, independently or co-inoculated in maize (*Zea mays* L.) and soybean (*Glycine max* L.). Eur J Soil Biol 45:28–35. <http://dx.doi.org/10.1016/j.ejsobi.2008.08.005>.
- Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57–67. <http://dx.doi.org/10.1023/A:1004326910584>.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. <http://dx.doi.org/10.1093/bioinformatics/bts199>.
- Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T, Sasamoto S, Watanabe A, Idesawa K, Iriguchi M, Kawashima K, Kohara M, Matsumoto M, Shimpo S, Tsuruoka H, Wada T, Yamada M, Tabata S. 2002. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Res 9:189–197. <http://dx.doi.org/10.1093/dnares/9.6.189>.
- Kaneko T, Maita H, Hirakawa H, Uchiike N, Minamisawa K, Watanabe A, Sato S. 2011. Complete genome sequence of the soybean symbiont *Bradyrhizobium japonicum* strain USDA6<sup>T</sup>. Genes 2:763–787. <http://dx.doi.org/10.3390/genes2040763>.
- Siqueira AF, Ormeño-Orrillo E, Souza RC, Rodrigues EP, Almeida LG, Barcellos FG, Batista JS, Nakatani AS, Martínez-Romero E, Vasconcelos AT, Hungria M. 2014. Comparative genomics of *Bradyrhizobium japonicum* CPAC 15 and *Bradyrhizobium diazoefficiens* CPAC 7: elite model strains for understanding symbiotic performance with soybean. BMC Genomics 15:420. <http://dx.doi.org/10.1186/1471-2164-15-420>.
- Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of standard operating procedures (SOPs) for (meta)genomic annotation. Omics 12:137–141. <http://dx.doi.org/10.1089/omi.2008.0017>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
- Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 35:W182–W185. <http://dx.doi.org/10.1093/nar/gkm321>.